Effect of mycorrhiza and phosphorus in growth and flowering of daffodil (*Narcissus pseudonarcissus* L.) growing in calcareous soil

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Abstract

The daffodil flower is one of the commercial ornamental plants in the world today for cut flowers and garden plants. The aim of the study is to determine the influence of arbuscular mycorrhiza fungi (*Glomus intraradices*) with phosphorus on growth of daffodil plants in calcareous soil. The application of arbuscular mycorrhiza fungi (AMF) with different levels of phosphorus (0, 40, 80, 120, and 160 kg ha⁻¹) significantly affected daffodil characteristics. The treated plants with AMF and 160 kg ha⁻¹ phosphorus (P) gave the superior spike length (49.00 cm), spike diameters (9.84 mm), flower diameter (57.76 mm), leaf number (8.33), leaf length (51.33 cm), chlorophyll intensity (136.78 SPAD), leaf dry matter (16.49 %), root number (67.33), root length (35.00 cm), nitrogen (17.90 g kg⁻¹), and potassium (19.23 g kg⁻¹). On the other hand, the plants were treated with AMF and 80 kg ha⁻¹ P resulted in the maximum root colonization (72 %), phosphorus content (13.23 g kg⁻¹), iron (345.67 mg kg⁻¹), and zinc (86.33 mg kg⁻¹) contents. In contrast, the minimum results in this study were found in non-inoculant plants with untreated phosphorus. Based on the results of this study, the application of AMF along with 160 kg ha⁻¹ P doses was recommended for improving the flower and growth characteristics of daffodil.

Key words: *Narcissus*; Ornamental Plants; Mycorrhiza; Nutrients; Spike length; Phosphorus


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Introduction

The cultivation of Ornamental flower bulbs is becoming more important as a floricultural plant [1]. Flower bulbs are a highly favored landscaping character that adds crucial design and color components. Their diversity of flower shapes, sizes, vibrant colors, and periods of flowering make them especially valued [2]. The daffodil (Narcissus pseudonarcissus L.) is a widely used flower bulb for ornamental purposes and is one of the most popular ones [3] including cut flowers, garden flowers, park and pot plants [4].

The quality of plants, such as their growth, flowering, and bulb productivity, is dependent on the right balance of macro and micronutrients that they can absorb and utilize effectively. In order to achieve this balance, it is important to ensure that the plants receive the appropriate nutrition [5]. Phosphorus is a crucial nutrient required for optimal plant growth and development, playing a vital role in plant production. The inadequate availability of phosphorus has emerged as a significant constraint, limiting contemporary agricultural productivity [6]. In Kurdistan region-Iraq, the soil has been categorized as calcareous due to its high CaCO₃ content, resulting in a slightly alkaline soil reaction (pH). The phosphorus availability is very low in calcareous soil due to its chemical and physical fixation [7], which also affected soil properties such as soil structure, water relations, and soil crusting, as well as nutrient availability for plants [8]. Several methods exist to enhance the availability of phosphorus and other nutrients in the soil while reducing soil and water. One important approach involves the symbiotic association between specific plants and microorganisms that can enhance soil fertility, promote plant growth and mineral nutrition, and mitigate ecological pollution [9]. Moreover, microbial inoculum biofertilizers such as arbuscular mycorrhizal fungi (AMF) can be employed. It is significantly enhanced plant growth and soil fertility properties by augmenting the absorption of nutrients and water [10]. The AMF exhibits symbiotic relationships with the majority of land plants and is environmentally sustainable. AMF significantly contributes to the uptake of plant phosphorus through the facilitation of root growth [11]. The hyphae of fungi absorb the insoluble phosphorus and convert it into the solubilized form which is taken up by the plant [12]. In general, there is a problem in the soil of the Sulaymaniyah government which contain a high amount of CaCO₃ which fixes the available phosphorus. Hence, the aim of this investigation was to improve plant morphology and mineral compositions of daffodil flower bulbs in calcareous soil via the application of biofertilizers and phosphorus concentrations.

Materials and Methods

Experimental Site and Plant Materials

This investigation was conducted during the 2021-2022 growing season at the Horticulture Department, College of Agricultural Engineering Sciences, University of Sulaimani, Kurdistan Region-Iraq, with a GPS reading of (35° 53’ 7.9” N, 45° 36’ 4.5” E). The study was carried out in a high tunnel (15 m length, 4 m width, 2 m height) covered with 200 μm polyethylene plastic film. The meteorological inside the high tunnel at the time of the experiment were summarized in Table 1.
Table 1. Some meteorological data inside the high tunnel during the study period.

<table>
<thead>
<tr>
<th>Month</th>
<th>Temperature (C)</th>
<th>Relative humidity %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>November</td>
<td>7.53</td>
<td>30.90</td>
</tr>
<tr>
<td>December</td>
<td>6.20</td>
<td>24.01</td>
</tr>
<tr>
<td>January</td>
<td>2.74</td>
<td>24.42</td>
</tr>
<tr>
<td>February</td>
<td>3.45</td>
<td>28.29</td>
</tr>
<tr>
<td>March</td>
<td>8.83</td>
<td>32.08</td>
</tr>
<tr>
<td>April</td>
<td>10.4</td>
<td>38.5</td>
</tr>
</tbody>
</table>

The experiment was started by planting the Daffodils (*Narcissus pseudonarcissus*) true bulbs in pots, each pot containing 4 kg of soil. The soil samples were used for the pots and taken at (15-30 cm) depth from an agricultural farm. The soil is air-dried and sieved by 4 mm, in order to determine the baseline soil properties. Soil characteristics are summarized in Table 1. The pots were arranged according to a Completely Randomized Design (CRD) layout with three replications. The replications were divided and arranged into 10 treatments. The phosphorus was applied with different levels (0, 40, 80, 120, and 160 kg p ha\(^{-1}\)) in the form of \(\text{KH}_2\text{PO}_4\), which were inoculated and non-inoculated before planting. Then, each inoculated pot was applied with arbuscular mycorrhizal fungi (*Glomus intraradices*) 80 grams per pot with a carrier. The true bulbs were put on the carrier to ensure appropriate contact of the roots with the mycorrhiza by soil application method (SA) according to [13].

They are planted. In each replication, one true bulb was planted in the pots.

Table 2. Some important physical and chemical properties of the soil used in the experiment.

<table>
<thead>
<tr>
<th>Soil properties</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td>44.60 g kg(^{-1})</td>
</tr>
<tr>
<td>Clay</td>
<td>480.20 g kg(^{-1})</td>
</tr>
<tr>
<td>Silt</td>
<td>475.20 g kg(^{-1})</td>
</tr>
<tr>
<td>Texture</td>
<td>Silty clay</td>
</tr>
<tr>
<td>pH</td>
<td>7.20</td>
</tr>
<tr>
<td>EC</td>
<td>0.36 dsm(^{-1})</td>
</tr>
<tr>
<td>Organic matter</td>
<td>9.10 g kg(^{-1})</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>245 g kg(^{-1})</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>0.10 g kg(^{-1})</td>
</tr>
<tr>
<td>Available phosphorus</td>
<td>3.15 g kg(^{-1})</td>
</tr>
<tr>
<td>Soluble potassium</td>
<td>0.38 g kg(^{-1})</td>
</tr>
<tr>
<td>Iron</td>
<td>3.10 mg kg(^{-1})</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.45 mg kg(^{-1})</td>
</tr>
</tbody>
</table>

**Data Collection**

During the study, several parameters were measured using different instruments. Chlorophyll intensity was measured using a Monitor chlorophyll meter (SPAD 502 PLUS). Shoot dry matter (DM %) was measured by taking the leaf fresh weight (wb) and dried at 65°C in a forced-air oven for 72 hr until reaching the constant weight. Then, it was weighed again (wa), and the dry weight percentage was calculated by the following equation. \(\text{DM}\% = \frac{\text{wa}}{\text{wb}} \times 100\)

Mineral compositions including nitrogen, phosphorus, potassium iron, and zinc, were quantified according to [14] using atomic absorption spectroscopy.
Statistical analysis
The measured data were analyzed using two-way ANOVA-CRD by XLSTAT software, version 2019.2.2. Duncan’s multiple ranges test at $P \leq 0.05$ was applied to compare the means.

Results and Discussion
The data in Figure (1) exhibited that the application of arbuscular mycorrhizal fungi (AMF) and P levels had a significant effect on the morphological flower quality; spike length, spike diameter, and flower diameter. The findings proved that AMF and P significantly increased spike length when compared to other treatments (Figure 1A). Accordingly, the longest spike (49 cm) was obtained from the plants inoculated with AMF and fertilized with P at 160 kg ha$^{-1}$. Inversely, the shortest spike (17.67 and 22.67 cm, respectively) was observed from non-inoculated and inoculated plants with AMF without P application. Also, AMF and P at 160 kg ha$^{-1}$ were significantly superior to all the other treatments in terms of spike diameter (9.84 mm) and flower diameter (57.76 mm). While the lowest results of spike diameter (4.97 mm) and flower diameter (32.00 mm) were recorded in the non-inoculated plants and without P fertilization (Figure 1B and 1C). The utilization of AMF in treated plants with P resulted in superior flower characteristics in comparison to non-inoculated plants. This may be attributed to the increase in the absorption of water and minerals [15]. According to [16], the combined application of biofertilizers and mineral fertilizers has a positive effect on the spike length and diameter of the Narcissus plants. [17] found that the increasing phosphorus concentrations significantly affect spike length. Moreover, [18] explained that the flower diameter was significantly enlarged by AMF. The reason for the plant’s vigorous growth and larger flowers could be linked to the increased nutrient levels and the presence of AMF. This causes photosynthates to be drawn towards the flowers, leading to an intensification of the sink and resulting in larger flower size [19] in gladiolus.

Figure 1: Effect of AMF and phosphorus concentrations on (A) spike length, (B) spike diameter, and (C) flower diameter of the daffodil flower. The same letter on the columns indicates no significant differences between means according to Duncan’s multiple range test at ($P \leq 0.05$).

Regarding the leaf attributing parameters of daffodil flower in Figure (2). The inoculated plant had a significantly high
leaf number. The AMF with P at 160 kg ha$^{-1}$ registered the greatest leaf number (8.33), but the minimum number of leaves (3.33) was obtained in non-inoculated plants with 0 kg ha$^{-1}$ of P (Figure 2A). Moreover, the effect of AMF inoculation and 160 kg ha$^{-1}$ P on the leaf length (51.33 cm) was more prominent compared with all other treatments (Figure 2B). Whereas, the 0 of P without using AMF gave the shortest leaf (16.67 cm). The interaction of AMF and the highest rate of P treatment significantly enhanced the number of leaves per plant. Similarly, [16] stated that the interaction between biofertilizers and high phosphorus doses significantly increased the number of leaves per plant.

Figure 2: Effect of AMF and phosphorus concentrations on (A) plant leaf number, and (B) leaf length of the daffodil flower. The same on the columns indicates no significant differences between means according to Duncan’s multiple range test at (P≤ 0.05).

Chlorophyll intensity and leaf dry matter of the daffodil flowers were affected by the application of AMF and P concentrations (Figure 3). The highest chlorophyll intensity (136.77 SPAD) was achieved in inoculated plant with 160 kg ha$^{-1}$ P (Figure 3A). However, the lowest intensity (68.60 SPAD) was recorded in non-inoculated plants with 0 kg ha$^{-1}$ P. With respect to dry matter of leaves (Figure 3B), AMF with P at 160 kg ha$^{-1}$ showed a maximum percentage (16.49 %), but the results decreased substantially in the plants without AMF and P (8.17 %), along with the plants with AMF but without P (10.01%). The augmentation in the daffodil plants’ leaf dry matter in response to increasing concentrations of phosphorus could potentially be attributed to the role of phosphorus in encouraging the growth of the root system in an extensive manner. This, in turn, enhances the plants’ capacity to uptake water and essential nutrients from the depth of the soil, subsequently resulting in higher production of assimilates and consequently increased biomass [20].
The results in Figure (4) showed the effect of AMF and various phosphate levels on the root system of the daffodil flower. So, root number has been enhanced by mycorrhizal inoculant and increasing rate of phosphorus. The maximum root number (67.33) was found in AMF with 160 kg ha$^{-1}$ P. While, it was significantly decreased (21.33) in non-inoculate plants with 0 kg ha$^{-1}$ P (Figure 4A). Furthermore, the longest roots (13.33 cm) were measured in the plant from the bulbs treated with AMF and 160 kg ha$^{-1}$ P (Figure 4B). Oppositely, non-inoculated bulbs with 0 kg ha$^{-1}$ P gave the shortest roots (13.33 cm). Moreover, the greatest root colonization (72%) was observed with AMF and 80 kg ha$^{-1}$ P applications. However, the smallest root colonization (12%) was recorded in untreated plants with AMF and P levels (Figure 4C). The association between mycorrhizal fungi and plants results in an overproduction of auxin beyond the optimum level. Consequently, the surplus auxin inhibits the growth of the root length while encouraging the development of lateral roots [21]. [22] explained that AMF and other microorganisms possess the capacity to generate enzymes that solubilize nutrients, thereby augmenting the assimilation of nutrient components. In addition, the fungal hyphae elongate towards immobile ions, leading to an expansion of the root surface area, which allows for access to a greater volume of soil, hence facilitating enhanced nutrient uptake [23]. In addition, applying high levels of phosphorus (P) has been found to reduce the colonization, abundance, and diversity of AMF communities in both the roots and soil. However, moderate levels of P application may actually enhance the diversity of AMF communities, while excessive application of P can lead to decreased diversity [24]. [25] mentioned that both AMF and phosphate were significantly increased the percentage of mycorrhizal colonization compared to either single inoculation or untreated plants.
Figure 4: Effect of AMF and phosphorus concentrations on (A) plant root number, and (B) root length, and (C) root colonization of the daffodil flower. The same letter on the columns indicates no significant differences between means according to Duncan’s multiple range test at (P ≤ 0.05).

As shown in figure (5) the data relating to macronutrient contents in the daffodil flower were beneficially affected by AMF and phosphorus applications. Nitrogen in shoots were the highest (17.90 g kg⁻¹) in the inoculated plants which were fertilized with P at 160 kg ha⁻¹ compared with other plants inoculated or non-inoculated under the rest of P doses (Figure 4A). Further, Nitrogen reached the lowest concentration (9.43 g kg⁻¹) in the plants without neither AMF nor P applications. In addition, the best phosphorus content (13.23 g kg⁻¹) was observed due to the application of AMF together with 80 kg ha⁻¹ P (Figure 4B). A sharp reduction of phosphorus content (5.62 g kg⁻¹) was quantified in untreated plants with AMF and P doses. Furthermore, the application of AMF and phosphorus highly significantly influenced increasing potassium content (Figure 5C). The highest K content (19.23 g kg⁻¹) was detected at AMF and 160 kg ha⁻¹ of P treatments. Whereas, the plants without AMF and P doses demonstrated the lowest K content (10.52 g kg⁻¹). AM fungal external mycelia may be responsible for the increased phosphorus uptake in mycorrhizal plants. The AM fungal external mycelia can cover a larger soil area, allowing for more phosphorus absorption, and the mycelium of AM fungi also helps to increase the number of root phosphate absorption sites, thus improving the plant’s ability to take up more phosphorus [26]. Mycorrhizal plants may have a higher concentration of potassium as a result of the ability of AMF to enhance plant growth and nutrient uptake, including potassium. The hyphae of AM fungi can absorb more potassium and contribute to the total root surface area, leading to greater uptake of potassium by the plant and an increase in potassium concentration in the plant [27]. Another reason for this could be the important role that soil microorganisms play in controlling the decomposing of organic matter and the availability of key plant nutrients, such as nitrogen, phosphorus, and [13].
Alongside of NPK content, iron, and zinc in the plant shoots were measured (Figure 6). In respect of iron, the highest value (345.67 mg kg\(^{-1}\)) was achieved in the inoculated plants with AMF and fertilized with 80 kg ha\(^{-1}\) P (figure 6A), followed by AMF with 40 kg ha\(^{-1}\) P (272.45 mg kg\(^{-1}\)). However, increasing phosphorus concentration to the highest level (160 kg ha\(^{-1}\)) decreased iron content. The lowest values (152.48 and 152.7 mg kg\(^{-1}\)) were found in the untreated plants with AMF and P doses together with non-inoculated plants under 160 kg ha\(^{-1}\) P, respectively. Moreover, the AMF with 80 kg ha\(^{-1}\) P resulted in the highest zinc content (86.33 mg kg\(^{-1}\)) compared with other treatments (Figure 6B). Meanwhile, the minimum value of zinc contents (48.02 mg kg\(^{-1}\)) was noted in the plants without AMF and P treatments. On the whole, elevating P concentration to 120 and 160 kg ha\(^{-1}\) declined iron and zinc content, whether they inoculated or non-inoculated with AMF. The use of AMF alters the uptake of Fe and Zn by the plant, depending on the chemical properties of the soil substrate and the effectiveness of the root system. In situations where the levels of Fe and Zn are low and the soil is alkaline, the colonization of the plant's roots by AMF enhances the absorption of these micronutrients [28]. Moreover, several researchers have reported that the AM fungi may be responsible for the rise in Fe levels in inoculated plants because they can enhance plant growth and nutrient absorption, including Fe, while, also having the capacity to absorb and transport Fe to the host plant through their hyphae. The AM fungi's capability to augment the total surface area of the roots and promote hyphae growth is responsible for absorbing more Fe into the plant, which ultimately leads to an increase in the Fe content of the shoot [29]; [27].
As shown in Figure (7), PCA1 and PCA2 were responsible for (94.30%) of the variations. PCA1 carried (80.88%) of the variables and PCA2 (13.42%). In this regard, P120×AMF situated at both positive sides of PCA1 and PCA2 and strongly grouped with spike diameters (SD), spike length (SL), and flower diameter (FD). Similarly, P80×AMF at the same position is firmly associated with phosphorus (P). At the same time, P160×AMF at the positive side of PCA1 favorably related to plant leaf number (PLN), leaf length (LL), leaf chlorophyll intensity (LCI), leaf dry matter (DM), plant root number (PRN), potassium (K), root length (RL), and nitrogen (N). However, P0, to a lesser extent P40 and P0×AMF, at both negative sides of PCA1 and PCA2 had an adverse link with most of the measurements. Also, a negative connection existed among P80 and P0×AMF with PLN, LL, LCI, DM, PRN, K, RL, and N. On the other hand, figures (7) and (8) confirmed that most studied parameters had a high positive correlation with each other (p ≤ 0.05) with exception of iron (Fe), zinc (Zn), and root colonization (RC). Besides, Zn positively correlated with Fe, and RC positively associated with Fe and Zn.

**Interconnections of the variables**
Figure 7: PCA biplot explaining the distributions of the researched AMF and phosphorus concentrations on spike length (SL), spike diameters (SD), flower diameter (FD), plant leaf number (PLN), leaf length (LL), leaf chlorophyll intensity (LCI), leaf dry matter (DM), plant root number (PRN), root length (RL), root colonization, nitrogen (N), phosphorus (P), potassium (K), iron (Fe), and zinc (Zn).

Figure 8: Pearson’s correlation analysis of the studied traits, (p=0.05). spike length (SL), spike diameters (SD), flower diameter (FD), plant leaf number (PLN), leaf length (LL), leaf chlorophyll intensity (LCI), leaf dry matter (DM), plant root number (PRN), root length (RL), root colonization, nitrogen (N), phosphorus (P), potassium (K), zinc (Zn), and iron (Fe).
Conclusion
This study indicated the significant influence of applied arbuscular mycorrhizal fungi (AMF) along with different concentrations of phosphorus (P) on the daffodil (Narcissus pseudonarcissus L.) flower. The morphological plant characteristics and mineral compositions including nitrogen and potassium were significantly influenced by AMF with the highest phosphorus concentrations (160 kg ha\(^{-1}\)). However, root colonization and other minerals such as phosphorus, iron, and zinc proved better in plants inoculated with AMF and fertilized with 80 kg ha\(^{-1}\) P. On the other hand, the lowest value of all attributing parameters in this study was achieved from untreated plants with AMF and P doses. On the basis of this experiment, it can be observed that the application of biofertilizers with phosphorus provides a great future for the beneficial effect in the growth establishment and flowering quality of daffodil plants.

References


تأثير المايكورايزا والفوسفور فى نمو وزهور النرجس

Narcissus pseudonarcissus L.

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المستخلص

تعتبر زهره النرجس (الدافوديل) احدي اهم نباتات الزينة التجارية على مستوى العالم لاستخدامها كأزهار قطف ونباتات الحدائق. هدفت هذه الدراسة لتحديد تأثير التلقح بفطر المايكورايزا (Glomus interaradices) وضافة الفوسفور على نمو نباتات النرجس في التربة الكلسية. أثر التلقح بفطر المايكورايزا مع مستويات مختلفة من الفوسفور (0, 40, 80, 120 و 160 كغم م-1) بشكل معنوي في خصائص نباتات النرجس. أعطت النباتات الملقحة بفطر المايكورايزا و 160 كغم م-1 أعلى قيمة معنوية في طول الحامل الزهري (49.00 سم)، قطر الحامل الزهري (9.84 مم)، قطر الزيرة (57.76 مم)، عدد الأوراق (8.33)، طول الورقة (136.78 سم)، المادة الجافة للأوراق (16.49 %)، عدد الجذور (67.33)، طول الجذر (74.00 مم)، النتروجين (17.90 غم كغم-1) والألكالومين (19.23 غم كغم-1). ونامى نسبة أخرى النباتات الملقحة بفطر المايكورايزا و 80 كغم م-1 من الفوسفور اعتبرت أعلى قيمة في كل من الاصابات الفطرية على الجذور (72 %)، محتوى الفوسفور (13.23 غم كغم-1)، حديد (67.67 مغ كغم-1) و محتوى الزنك (86.33 مغ كغم-1). وفي المقابل أدنى القيم المسجلة كانت في النباتات الغير ملقحة بفطر المايكورايزا و الغير مضافة للإبس فوسفور، نداء على النتائج يوصي باللقاح بفطر المايكورايزا وضافة 160 كغم م-1 لتحسين خصائص الزهار والنمو الحضري لنباتات النرجس.

الكلمات المفتاحية: نرجس؛ نباتات الزينة؛ الفطريات؛ العناصر الغذائية؛ طول السبورة؛ الفوسفور.